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Synthesis and evaluation of antioxidant and antiproliferative activity of 2-arylbenzimidazoles

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ABSTRACT

Three series of arylbenzimidazole derivatives **3–40, 45** have been simply synthesized and tested for their antioxidant capacity. The 2-arylbenzimidazoles were tested against various radicals by the DPPH, FRAP and ORAC tests and showed different activity profiles. It has been observed that the number and position of the hydroxy groups on the 2-aryl portion and the presence of a diethylamino group or a 2-styryl group are related to a good antioxidant capacity. Furthermore, benzimidazoles showed satisfactory SPF values *in vitro* compared to the commercial PBSA filter, proving to have a good photoprotective profile. In particular, 2-arylbenzimidazole-5-sulphonic acids **15** and **38,** the 2-styryl-benzimidazole **45** showed broad spectrum solar protection against UVA and UVB rays. The antiproliferative effect of the benzimidazoles was tested on human skin melanoma Colo-38 cells. The styrylbenzimidazole **45** exhibited antiproliferative effect at low micromolar concentration against Colo-38 cells and very low antiproliferative activity on normal HaCat keratinocyte cells.

1. Introduction

Reactive oxygen (ROS) and nitrogen (NOS) species produced by metabolism of endogenous and exogenous substances led to oxidative stress. Clinical evidences account for the involvement of oxidative stress-induced by reactive oxygen and nitrogen species in a variety of disorders including cancer [1], atherosclerosis [2] neurodegeneration and aging [3]. ROS can be generated by UVB and UVA radiation producing protein oxidative modification resulting in photoaging. ROS cause tissue modification that at skin level is elicited by the loss of the barrier function of the stratum corneum, the promotion of inflammatory processes, erythema, up to the cancer [4,5]. The benzimidazole moiety represents a privileged structure in medicinal chemistry. Molecules of natural origin play a fundamental role in the research of bioactive molecular models for the development of new synthetic and semi-synthetic analogues. The discovery of 5,6-dimethyl-1-(D-ribofuranosil)-benzimidazole, as an integral part of vitamin B12, has generated considerable interest in the benzimidazole nucleus [6]. This can be considered as a structural isostere of indole and purines, or other natural active substances capable of interacting with other biomolecules, such as proteins and nucleic acids [7].

Benzimidazole is a pharmacophore that has found widespread application in medicinal practice. The benzimidazole ring system exists in the structure of many drugs such as the antihypertensive Candesartan, the proton pump inhibitor Pantoprazole, the antihistaminic Astemizole, the antimicrobial Albendazole, analgesics, anti-inflammatories, anticonvulsant, anti-psychotic, anti-diabetic, anthelmintic, antiprotozoal drugs and antifungals [8,9]. Literature data also report a remarkable antiproliferative, anti-tumor, and antiviral biological activity [10] of the various derivatives of the benzimidazole nucleus. Furthermore, benzimidazole derivatives have been reported for their antioxidant activity [11–14].

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The benzimidazole core also appears in some sunscreens; it is contained, for example, in 2-phenyl-1*H*-benzimidazole-5-sulfonic acid (PBSA), one of the UVB filters mostly used in cosmetics for sun protection. PBSA is characterized by high water solubility in and has an excellent safety profile. Another effective example is the disodium salt of phenyl-dibenzimidazole-tetrasulfonate (known as Neo Heliopan® AP) which absorbs mainly in the UVA range. Neo Heliopan® AP is soluble in aqueous phase when added with a base, it is stable and safe, with an extremely low degree of penetration of the skin. In this context, a molecule was designed and synthesized with the aim of combining the filtering capacity of the already known PBSA and the strong antioxidant properties of polyphenols. The final product, called Oxisol (2-(3,4-dihydroxy-phenyl)-1*H*-benzimidazole-5-carboxylic acid), is not only a powerful antioxidant and absorbs in the UVA range, but is also equipped with SPF booster effect [15–16]. As a continuation of our efforts to identify dualistic molecules [17,18] able to act, at the same time, as antioxidant and photoprotective agents here we describe the design, synthesis and biological evaluation of three series of benzimidazoles differing for the substituent at 5-position and bearing a variety of hydroxyaryl moieties at 2-position.

2. Results and discussion

2.1. Chemistry

The target benzimidazoles **3–40, 45** (Table 1) were synthesized as shown in Schemes 1 and 2 starting from readily available and inexpensive starting materials. The ethanolic solution of diaminobenzenes **1a–d** was added with sodium metabisulphite aqueous solution and the appropriate aldehyde **2**. The resulting solution was refluxed to achieve benzimidazoles (**3–40**) in 52–88% yields (Scheme 1). Benzimidazole **45** was obtained following the reaction sequence showed in Scheme 2. 4-Hydroxycinnamic acid (**41**) was coupled with methyl 3,4-diaminobenzoate (**42**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and hydroxybenzotriazole (HOBt) in anhydrous acetonitrile solution to give the intermediate **43**. Benzimidazole ring closure was performed in hydrochloric acid to give the ester derivative **44**. Compound **44** was submitted to mild basic hydrolysis to afford the benzimidazole (**45**). Structures of all new compounds were confirmed based on analytical and spectral data which are consistent with results of reported studies [15,19–21].

2.2. Antioxidant activity

The valuation of the antioxidant properties of the benzimidazoles **3–40, 45** was achieved by 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Oxygen radical absorbance capacity (ORAC) methods. Results are shown in Table 1 and are expressed as μmolTE/g for DPPH, FRAP, and ORAC tests. For the best interpretation of the results of the DPPH, each compound was tested at the concentration capable of inhibiting 50% of the radical. However, in some cases it was not possible to obtain data reflecting 50% inhibition, even if the tested compounds are all in the standard calibration line.

DPPH result analysis indicated that high antioxidant capacity is correlated to high number of hydroxy groups present on the aryl ring at the 2-position. Benzimidazole derivatives **3–11** bearing a single hydroxy group on the aryl ring showed weak activity independently from the substituent present at 5-position. The introduction of a second (compounds **13–17**) or a third hydroxy group (compounds **31–36**) led to the best compounds of the series. Nevertheless, the hydroxy group position also affects antioxidant activity: in fact, 2,5-dihydroxybenzimidazole derivatives **13, 14** were about 2 times more active than the 3,4-dihydroxyphenyl analogs **16, 17**. While the shift of the 3-OH of compound **16** into 2-position to give the analog **12** produced drop in activity. Furthermore, the 2,3,4-trihydroxyphenyl derivatives **31** and **32** were more active than the 2,4,6-trihydroxyphenyl analogs **34** and **35**. On the contrary the comparison of the 5-sulfonyl substituted benzimidazoles indicated that the 2,4,6-trihydroxyphenyl analog **36** was characterized by antioxidant activity better than the corresponding 2,3,4-trihydroxyphenyl derivative **33**. The replacement of one hydroxy group with an alkoxy group (compounds **18–24**) or halogen atoms (compounds **25–30**) led to reduction in activity. The introduction of a 4-diethylamino group (benzimidazoles **37** and **38**) produced increase in activity as compared to compounds **7** and **8**.

On FRAP analysis, according to the results obtained from the DPPH test the most powerful derivatives were benzimidazoles **13–17** and **31–37**. Interestingly, the 2-hydroxy-3-ethoxyphenyl derivatives **21, 22**, the 2-hydroxy-4-(diethyl)amino derivatives **38,** and the 4-hydroxystyryl derivative **45** demonstrated good activity.

The compounds that showed the most interesting profile following the DPPH and FRAP tests were also subjected to a further test (ORAC) to determine their complete antioxidant activity profile. The 4-hydroxystyryl derivative **45** demonstrated the best antioxidant capacity. The 2-hydroxy-4-(diethyl)aminophenyl derivatives **37** and **38** demonstrated high antioxidant capacity. Contrary to DPPH and FRAP assays, in ORAC test the 2,3,4-trihydroxyphenyl derivative **31** showed better activity as compared to the corresponding 2,4,6-trihydroxyphenyl analog **34**. The 5-COOH-benzimidazole derivatives (**32, 35**) and 5-SO_3H-benzimidazole (**33, 36**) analogs displayed a reverse trend in ORAC antioxidant results.

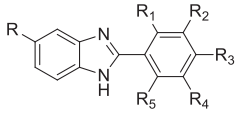
2.3. Evaluation of filtering parameters

On the benzimidazoles **13–17, 21, 22, 31–38, 40** and **45** endowed with the best antioxidant profile, *in vitro* tests were performed to determine the parameters fundamental for the evaluation of the filtering power: critical wavelength (λc) and SPF (Sun Protection Factor) and results are reported in Table 2. The Solar Protection Effectiveness Evaluation System specifies a SPF primarily representing a measure of UVB protection [22] is related to the UV absorption of substances. The UV spectra of the compounds **13–17**, **21**, **22**, **31–38**, **40** and **45** were recorded between 290 and 400 nm and compared with the commercial filter PBSA used as a reference UVB filter (λmax 302 nm). The superimposed UV spectra of the analyzed compounds were subdivided according to kind of substituent at 5-position (Figs. 1–3). The UV absorption spectra of 5-cyanobenzidimidazoles **13**, **16**, **31** and **34** (Fig. 1) showed λmax shifted towards longer wavelengths as compared to the reference PBSA. Compound **13** showed the λmax closest to PBSA; however, its absorption range was the widest in the series with an absorbance reaching zero near 400 nm. The bathochromic shift is correlated to the increase in the number of hydroxy groups on the 2-aryl ring: the wavelength of maximum absorption was in fact greater for derivatives **31** and **34**, both bearing three hydroxy groups. As can be seen from Fig. 2, 5-carboxylic acid benzimidazole derivatives **14**, **17**, **21**, **32**, **35**, **37** and **45** were also characterized by a wide range of absorption which is exhausted well over 400 nm. The spectral profile of benzimidazoles **37** and **45** was characterized by λmax shifted to wavelengths higher than 350 nm. However, all the 5-carboxylic acid benzimidazole derivatives displayed absorption spectra better than PBSA.

The profile of the UV spectra of the 5-sulfonic acid benzimidazole derivatives **15**, **22**, **33**, **36**, **38**, and **40** (Fig. 3) was again wider than the reference, furthermore the λmax were greater than PBSA. The bathochromic shift appeared particularly evident for compound **38**: once again, the presence of a tertiary amino group on the aromatic ring promotes the shift of the maximum absorption peak to the right in the spectrum.

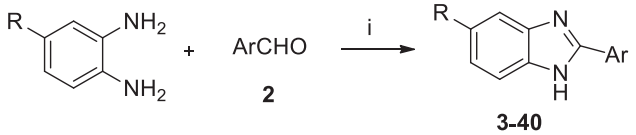
A preliminary *in vitro* study was carried out on the filtering properties to determine the UV protection potential of benzimidazoles **13–17, 21, 22, 31–38, 40** and **45**. To date there is an approved and standardized method for *in vitro* assessment of UVA protection present

Table 1
Antioxidant activity of the indole derivatives **3–40** and **45**.



Compound	R	R_1	R_2	R_3	R_4	R_5	DPPH[a] µmolTE/g	FRAP[a] µmolTE/g	ORAC[a] µmolTE/g
3	H	H	H	OH	H	H	10.9 ± 1.5	15.1 ± 0.4	–
4	CN	H	H	OH	H	H	< 15.8[b]	30.3 ± 2.7	–
5	COOH	H	H	OH	H	H	< 39.2[b]	94.2 ± 2.5	–
6	SO_3H	H	H	OH	H	H	no activity[c]	24.6 ± 1.8	–
7	COOH	OH	H	H	H	H	51.8 ± 0.6	103.3 ± 3.7	–
8	SO_3H	OH	H	H	H	H	no activity[c]	187.4 ± 4.9	–
9	CN	H	OH	H	H	H	< 18.3[b]	29.0 ± 2.5	–
10	COOH	H	OH	H	H	H	87.4 ± 1.2	223.4 ± 3.8	–
11	SO_3H	H	OH	H	H	H	no activity[c]	56.5 ± 1.2	–
12	CN	OH	H	OH	H	H	< 27.5[b]	209.2 ± 1.8	–
13	CN	OH	H	H	OH	H	4747.2 ± 19.9	10109.0 ± 13.7	20827.9 ± 29.5
14	COOH	OH	H	H	OH	H	4824.7 ± 11.6	10318.2 ± 15.8	18378.7 ± 18.4
15	SO_3H	OH	H	H	OH	H	515.7 ± 1.8	1562.7 ± 13.1	16014.2 ± 67.4
16	CN	H	OH	OH	H	H	2042.1 ± 7.3	6353.8 ± 11.6	8210.2 ± 25.0
17	COOH	H	OH	OH	H	H	1946.8 ± 8.1	5502.3 ± 13.2	13536.6 ± 15.6
18	CN	H	OH	OMe	H	H	39.9 ± 0.9	72.7 ± 3.3	–
19	COOH	H	OH	OMe	H	H	48.4 ± 3.5	70.1 ± 2.3	–
20	SO_3H	H	OH	OMe	H	H	no activity[c]	814.9 ± 5.8	–
21	COOH	OH	OEt	H	H	H	109.7 ± 0.4	4098.7 ± 16.3	7639.9 ± 25.9
22	SO_3H	OH	OEt	H	H	H	< 50.9[b]	2556.6 ± 17.6	13900.6 ± 61.4
23	COOH	OH	H	OMe	H	H	41.2 ± 2.4	113.5 ± 5.1	–
24	SO_3H	OH	H	OMe	H	H	no activity[c]	27.7 ± 2.4	–
25	CN	OH	H	H	Cl	H	45.0 ± 1.8	64.7 ± 3.9	–
26	COOH	OH	H	H	Cl	H	81.6 ± 3.4	94.2 ± 2.5	–
27	SO_3H	OH	H	H	Cl	H	≪59.6[b]	174.7 ± 6.2	–
28	CN	OH	H	H	Br	H	48.7 ± 1.7	48.5 ± 1.5	–
29	COOH	OH	H	H	Br	H	81.3 ± 0.9	117.3 ± 4.1	–
30	SO_3H	OH	H	H	Br	H	≪50.0[b]	63.4 ± 2.7	–
31	CN	OH	OH	OH	H	H	7112.7 ± 15.1	12049.2 ± 19.2	8312.9 ± 31.8
32	COOH	OH	OH	OH	H	H	5026.6 ± 13.9	11375. ± 19.6	7321. ± 17.8
33	SO_3H	OH	OH	OH	H	H	1324.1 ± 14.2	9097.5 ± 16.7	6726.9 ± 17.4
34	CN	OH	H	OH	H	OH	1473.0 ± 10.3	7138.2 ± 12.4	4758.0 ± 13.7
35	COOH	OH	H	OH	H	OH	897.3 ± 5.6	2334.8 ± 7.5	9467.8 ± 63.2
36	SO_3H	OH	H	OH	H	OH	5026.6 ± 13.8	1970.3 ± 3	8679.5 ± 28.0
37	COOH	OH	H	$N(Et)_2$	H	H	165.1 ± 7.5	5233.1 ± 6.2	14583.9 ± 110.0
38	SO_3H	OH	H	$N(Et)_2$	H	H	84.9 ± 0.7	925.7 ± 8.8	12871.7 ± 35.6
39	COOH	2-OH-naphthyl					< 27.6[b]	187.7 ± 3.1	–
40	SO_3H	2-OH-naphthyl					4.9 ± 0.7	527.7 ± 8.8	4297.5 ± 41.5
45	COOH	4-OH-styryl					31.33 ± 0.7	792.0 ± 5.0	24852.0 ± 28.5

[a] Each value was obtained from three experiments (mean ± SE).
[b] LOQ limit of quantification; – not tested.
[c] Precipitation of the compound is observed.

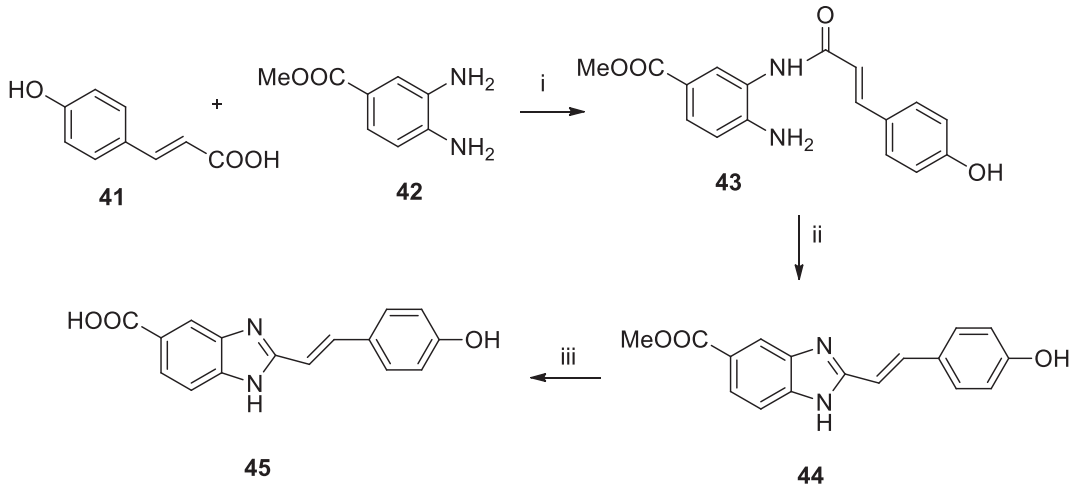


1a R = H
1b R = CN
1c R = COOH
1d R = SO_3H

Scheme 1. Reagents and conditions: EtOH, 2.5 N sodium metabisulphite, reflux 24 h.

in ISO 24443: 2012, however for the evaluation of UVB protection there is still no approved *in vitro* method. The method proposed by Diffey-Robson for the *in vitro* evaluation of the SPF parameter in 1989 [23] is still applied. The UV absorption spectra of the benzimidazoles **13–17, 21, 22, 31–38, 40** and **45** as well as the reference PBSA were recorded and the values relating to the filtering parameters were extrapolated (Table 2). According to the EU recommendation on the efficacy of sun protection products (2006/247/EC) a broad-spectrum solar product, capable of protecting the skin simultaneously from UVA and UVB rays, should have a λc value greater than 370 nm. On this criterion, the only interesting compounds of the 2-aryl-benzimidazole series were derivatives **15** and **38** bearing a sulfonic acid moiety and the 2-styryl-benzimidazole **45**. Another parameter that provides indications on the best blocked UV region is the UVA/UVB absorbance ratio. As defined by the aforementioned EU recommendation (2006/247 / EC), this ratio should be worth at least 1/3. Almost all the compounds belonging to the three series satisfied this requirement, most of them with values greater than 1, to indicate an absorption mainly in the UVA region.

Finally, the value of UVAPF0 was determined in relation to the guidelines provided by ISO-24443: all the above-mentioned polyhydroxylated compounds, which meet the effectiveness requirements imposed by EU legislation, have a better UVA Protection Factor than the reference commercial filter. The most interesting data were showed by compounds **37** and **38**, with a UVAPF0 value of 14.30 and 15.77 respectively.



Scheme 2. Reagents and conditions: (i) EDC, HOBt, MeCN, r.t. 36H; (ii) EtOH, 6 N HCl, reflux 5 h; (iii) EtOH, 2.5 N NaOH, r.t. 24 h.

2.4. In vitro release study of topical formulations

In analogy with one of our previously reported studies [18], we selected the most promising candidate in terms of antioxidant capacity and UV-filtering profile, compound **15**, to assess its potential as an active ingredient of topical formulations *in vitro*. Therefore, the formulation conceived and designed for Oxisol [15], a molecule with dualistic activity, has been optimized for compound **15**. Two topical formulations were then prepared with different degrees of polarity on the basis of the structures of the two compounds: Formulation (A) to obtain a high release of the active substance that carries out activity after absorption in the skin (active acting as an antioxidant) and the Formulation (B) to obtain a limited release, through a better solubilization of the active principle in the formulation (active that acts as a solar filter). The release of the two ingredients from the topical formulations was evaluated through the Franz cell system. The Franz cells consist of two vertical chambers (donor and acceptor) separated by a synthetic or biological membrane which has both the support function and the means of separation between the receiving medium and the formulation for which the release is to be studied. In this study, both for compound **15** and for Oxisol, the receptor phase was sampled within 6 h to measure the quantity in the receiving chamber, the resulting release curves are reported in Fig. 4. It was observed a 0.8% and 7% difference between the release curves of the two different formulations that characterize Oxisol and compound **15**. Specifically, the two curves maintained the same trend as regards Oxisol, with a reduction in the delta after 240 min. With regard to derivative **15**, in the first 30 min the same release could be observed from formulations A and B, but in later times the gap between the two curves tends to increase until it reached a difference of 7%. These results, like those obtained previously [18], confirm that it is possible, only by varying the components percentages in the formulation, to modify the release of the active ingredient. This approach therefore consists of modifying the polarity of a formulation on the basis of both the activity and the end use of the chosen active ingredient.

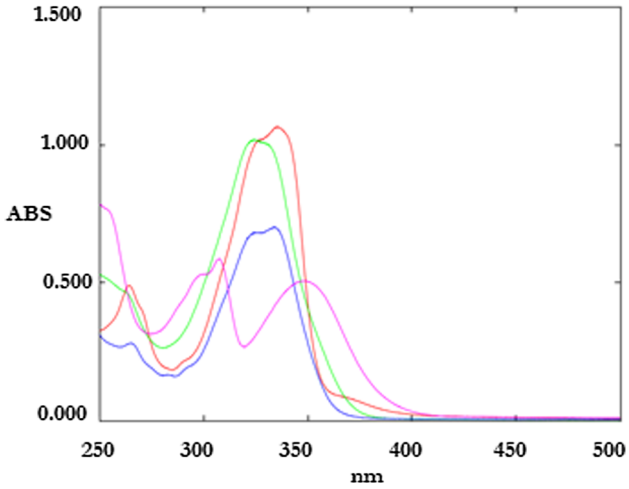


Fig. 1. UV absorption spectra of 5-cyano-2-arylbenzimidazole derivatives **13** (fuchsia), **16** (green), **31** (blue), **34** (red).

2.5. Antiproliferative activity on human melanoma Colo38 cell line

The benzimidazoles **13–17, 21, 22, 31–38, 40** and **45** were tested on human melanoma Colo38 cell line to determine their antiproliferative activity. All the compounds have been tested on the cell line to evaluate the relative IC_{50} values expressed in μM concentration (Table 3). Benzimidazole **45** exhibited the best activity of the series,

Table 2
UV-filtering activity of selected benzimidazoles in solution.

Compound	SPF	UVA/UVB	UVAPF0	λc (nm)	Compound	SPF	UVA/UVB	UVAPF0	λc (nm)
PBSA	3,4	0,29	1,03	322	**32**	2,71	1,04	1,51	348
13	2,93	0,73	2,28	369	**33**	1,70	0,81	1,10	343
14	2,79	0,75	2,19	368	**34**	3,34	1,27	1,78	349
15	2,55	0,70	2,06	373	**35**	3,20	0,90	1,49	355
16	4,63	0,86	1,79	350	**36**	4,96	0,67	1,58	350
17	4,26	0,85	1,71	349	**37**	1,79	2,10	14,30	387
21	6,03	0,37	1,39	342	**38**	1,71	2,20	15,77	386
22	6,25	0,29	1,32	340	**40**	2,3	0,77	1,62	362
31	2,56	1,10	1,48	347	**45**	2,42	2,27	7,86	384

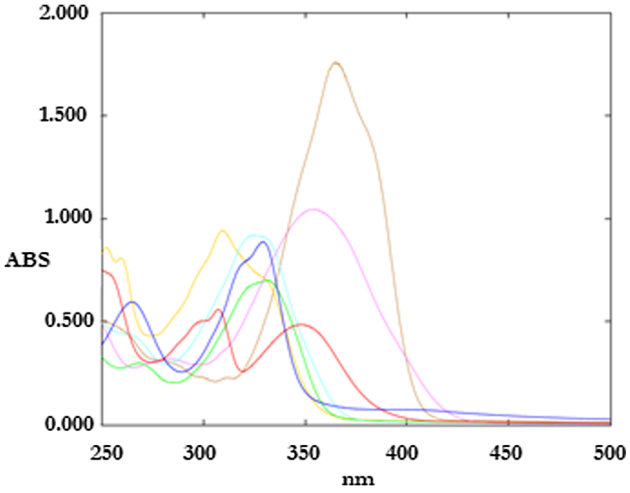


Fig. 2. UV absorption spectra of the 5-carboxylic acid benzimidazole derivatives **14** (red), **17** (blue), **21** (yellow), **32** (light green), **35** (blue), **37** (brown), **45** (pink).

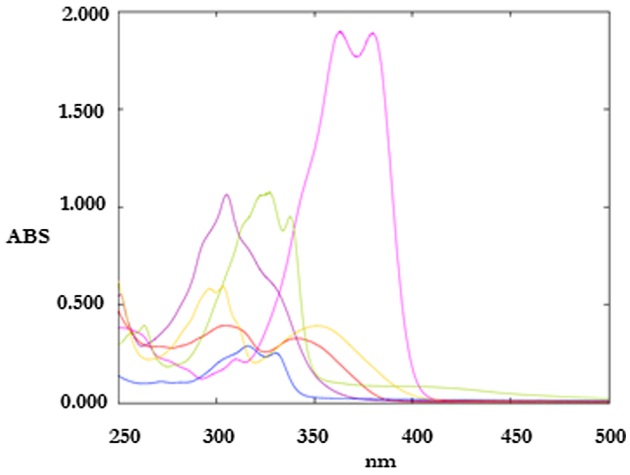


Fig. 3. UV absorption spectra of the 5-sulfonic acid benzimidazole derivatives **15** (yellow), **22** (purple), **33** (blue), **36** (green), **38** (fuchsia), **40** (red).

displaying an antiproliferative effect at low micromolar concentration (IC_{50} values 6.20 ± 0.22). The 2,3,4-trihydroxyphenyl derivatives **31–33** showed antiproliferative activity at micromolar concentration. On the contrary their 2,4,6-trihydroxyphenyl analogs (**34–36**) showed very poor antiproliferative effects or total inactivity at the used concentrations. Furthermore the 2,5-dihydrophenyl derivatives **13** and **15** inhibited the Colo38 cell growth at micromolar concentrations.

2.6. Antiproliferative activity on human keratinocyte HaCat cell line

The benzimidazoles **13** and **45**, demonstrating the best antiproliferative activity (IC_{50} values: 50.14 ± 2.41 and 6.20 ± 0.22, respectively) on the melanoma Colo38 cells, were also tested on normal human keratinocyte HaCat cell line to preliminary evaluate their selectivity against cancer cells. Both compounds **13** and **45** showed low activity against HaCat cells (IC_{50} values: 278.97 ± 48.01 μM and 32.72 ± 8.54 μM, respectively) being about 5-fold more active against Colo38 cell line. These results suggest a preferential activity of tested compounds against cancer cells.

3. Conclusion

A series of 2-arylbenzimidazole derivatives were synthesized with the aim to obtaining molecules with a dualistic functional profile, specifically in the context of multiprotective agents able to act both against the damage caused by the sun radiations exposure and by the oxidative stress promoted by free radicals.

All the synthesized benzimidazole derivatives **3–40, 45** were tested to evaluate their antioxidant profile with the DPPH and FRAP methods. The antioxidant activity profile was completed with the ORAC method only for the compounds that emerged as the best from the two above-mentioned tests. In general, it has been observed that the presence of a sulfonic acid group at 5-position of the benzimidazole nucleus is the least favourable, while the derivatives bearing a 5-cyano or 5-carboxyl acid groups have showed medium to high antioxidant activity.

The best antioxidant compounds were investigated for their UV-filtering activity showing heterogeneous profiles. Compounds **15**, **38** (bearing a sulfonic acid group), and the 2-styryl-benzimidazole **45** were the best in terms of broad spectrum filtering activity. All the analyzed benzimidazoles showed a better UVAPF0 value than the reference PBSA. The derivative **15** maintained its filtering profile even when incorporated into formulations for topical use and might be chosen as a lead compound for further development. Thus, its release was evaluated from two different formulations designed *ad hoc*, varying the

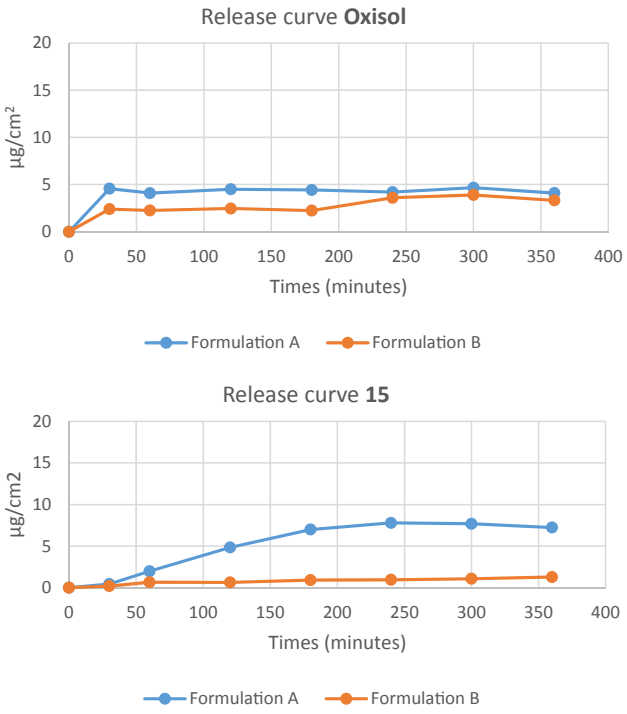


Fig. 4. Permeation profiles of Oxisol, and benzimidazole **15**. Line blue corresponds to formulation A (optimized for skin adsorption), line orange corresponds to Formulation B (optimized to best solubilize the active in formula).

Table 3
Effects of selected benzimidazole derivatives on the proliferation of Colo38 cells.

Compound	IC_{50} (μM)	Compound	IC_{50} (μM)
13	50.14 ± 2.41	**33**	74.4 ± 6.27
14	409.31 ± 4.44	**34**	446.96 ± 33.71
15	79.13 ± 10.21	**35**	> 500
16	62.02 ± 7.76	**36**	> 500
17	424.53 ± 44.47	**37**	171.84 ± 38.24
21	318.06 ± 40.49	**38**	450.79 ± 41.32
22	> 500	**40**	323.78 ± 22
31	96.23 ± 10.25	**45**	6.20 ± 0.22
32	65.07 ± 0.18		

composition only, to optimize the skin absorption or the solubility of the active compound to promote application of the formulations as an antioxidant or sun filter. Furthermore, the investigation on the antiproliferative activity on human melanoma Colo38 cells showed compound **45** as the best in the series with an IC_{50} value at low micromolar concentrations level. The same compound inhibited the growth of normal HaCat keratinocytes at higher concentrations demonstrating an interesting selectivity against cancer cells. The overall results obtained from this study have pointed out the key role played by the arylhydroxy group in the benzimidazole ring which led to compounds endowed with multifunctional profile, more specifically marked in antioxidant and UV filtering activity such us **15** and **45** that constitute interesting lead compounds.

4. Experimental section

4.1. General methods

All commercially available solvents and reagents were used without further purification. Standard samples were purchased from Sigma-Aldrich, Milan, Italy. NMR spectra were recorded on an Inova 500 spectrometer (Varian, Palo Alto, CA, USA). The chemical shifts (δ) are reported in part per million downfield from tetramethylsilane (TMS), which was used as internal standard, and the spectra were recorded in hexadeuteriodimethylsulphoxide (DMSO-d_6). Infrared spectra were recorded on a Vector 22 spectrometer (Bruker, Bremen, Germany) in Nujol mulls. The main bands are given in cm^{-1}. Positive-ion electrospray ionization (ESI) mass spectra were recorded on a double-focusing MAT 95 instrument (Finnigan, Waltham, MA, USA) with BE geometry. Melting points (mp) were determined on a SMP1 Melting Point apparatus (Stuart Scientific, Stone, UK) and are uncorrected. All products reported showed NMR spectra in agreement with the assigned structures. The purity of the tested compounds was determined by combustion

elemental analyses conducted by the Microanalytical Laboratory of the Chemistry Department of the University of Ferrara with a MT-5 CHN recorder elemental analyzer (Yanagimoto, Kyoto, Japan) and the values found were within 0.4% of theoretical values. The spectrophotometer used for antioxidant analysis is a Beckman Coulter™, DU®530, Life Science UV/VIS spectrophotometer, Single Cell Module. The instrument used to conduct ORAC analyzes is the Thermo Fluoroskan Ascent FL® Microplate Fluorometer and Luminometer, linked to Ascent Software® software for data control and processing. In the sample loading phase, 96-well plates with a black background were used. Spectrophotometric analysis for the detection of filter parameters were conducted with a UV–VIS spectrophotometer SHIMADZU UV-2600 240 V.

4.2. Chemistry

4.2.1. General procedure for the synthesis of benzimidazoles (3–40)

To a solution of the appropriate 3,4-diaminobenzene derivative (1a-d) (2 mmol) in ethanol (15 mL) 2.85 N aqueous solution of sodium metabisulphite (1.6 mL) and the appropriate substituted arylaldehyde (2 mmol) were added. The reaction mixture was heated at reflux for 24 h. The solvent was then evaporated under reduced pressure. The residue was added with HCl 1 N (10 mL), the formed precipitate was filtered off, washed with water (3 × 10 mL) and purified by crystallization from the adequate solvent to give the title compounds. Following the general procedure benzimidazoles **3** [19], **4** [20], **5** [21], **7** [24], **6**, **32** and **33** [15] were prepared and their analytical and spectral data are in agreement with those reported in literature.

4.2.1.1. 2-(3-Hydroxyphenyl)-1H-benzo[d]imidazole-5-carbonitrile (9). Yield 80%. Mp > 250 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.08 (d, J = 8.0 Hz, 1H, Ar), 7.42 (t, J = 8.0 Hz, 1H, Ar), 7.64–7.70 (m, 3H, Ar), 7.81 (d, J = 8.5 Hz, 1H, BIM), 8.19 (s, 1H, BIM). IR (Nujol) 2227, 1589 cm^{-1}. *m/z* 236 $(M+H)^+$. Anal. Calcd for $C_{14}H_9N_3O$: C, 71.48; H, 3.86; N, 17.86. Found: C, 71.41; H, 3.87; N, 17.90.

4.2.1.2. 2-(3-Hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (10). Yield 62%. Mp 208–210 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.03 (d, J = 7.5 Hz, 1H, Ar), 7.44 (t, J = 8.0 Hz, 1H, Ar), 7.64 (m, 2H, Ar), 7.74 (d, J = 8.5 Hz, 1H, BIM), 7.94 (d, J = 8.0 Hz, 1H, BIM), 8.23 (s, 1H, BIM), 9.96 (s, 1H, OH). IR (Nujol) 3360, 2726, 1693, 1569 cm^{-1}. *m/z* 255 $(M+H)^+$. Anal. Calcd for $C_{14}H_{10}N_2O_3$: C, 66.14; H, 3.96; N, 11.02. Found: C, 66.09; H, 3.98; N, 11.05.

4.2.1.3. 2-(3-Hydroxyphenyl)-1H-benzo[d]imidazole-5-sulfonic acid (11). Yield 69%. Mp 219–220 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.16 (d, J = 8.0 Hz, 1H, Ar), 7.53 (d, J = 8.0 Hz, 1H, Ar), 7.57 (s, 1H, Ar), 7.62 (d, J = 8.0 Hz, 1H, Ar), 7.77 (d, J = 8.0 Hz, 1H, Ar), 7.82 (d, J = 8.5 Hz, 1H, Ar), 9.96 (s, 1H, Ar), 10.23 (s, 1H, OH). IR (Nujol) 3397, 3274, 1631, 1588 cm^{-1}. *m/z* 291 $(M+H)^+$. Anal. Calcd for $C_{13}H_{10}N_2O_4S$: C, 53.79; H, 3.47; N, 9.65. Found: C, 53.84; H, 3.46; N, 9.68.

4.2.1.4. 2-(2,4-Dihydroxyphenyl)-1H-benzo[d]imidazole-5-carbonitrile (12). Yield 63%. Mp 233–234 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 6.56 (d, J = 8.5 Hz, 1H, Ar), 6.68 (s, 1H, Ar), 7.81 (d, J = 8.0 Hz, 1H, BIM), 7.89 (d, J = 8.0 Hz, 1H, BIM), 8.05 (d, J = 8.5 Hz, 1H, Ar), 8.20 (s, 1H, BIM). IR (Nujol) 3348, 3211, 3086, 2242, 1611 cm^{-1}. *m/z* 252 $(M+H)^+$. Anal. Calcd for $C_{14}H_9N_3O_2$: C, 66.93; H, 3.61; N, 16.73. Found: C, 66.99; H, 3.60; N, 16.70.

4.2.1.5. 2-(2,5-Dihydroxyphenyl)-1H-benzo[d]imidazole-5-carbonitrile (13). Yield 78%. Mp > 250 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 6.98 (s, 2H, Ar), 7.50 (s, 1H, Ar), 7.73 (d, J = 8.0 Hz, 1H, BIM), 7.86 (d, J = 8.5 Hz, 1H, BIM), 8.21 (s, 1H, BIM). ^{13}C (DMSO-d_6): δ 108.2, 112.6, 116.6, 121.1 (2C), 125.6 (2C), 128.9, 130.3, 135.7, 138.9, 141.8, 147.1, 148.1. IR (Nujol) 2230, 1617, 1564 cm^{-1}. *m/z* 252 $(M+H)^+$. Anal. Calcd for $C_{14}H_9N_3O_2$: C, 66.93; H, 3.61; N, 16.73. Found: C, 66.87; H, 3.63; N, 16.77.

4.2.1.6. 2-(2,5-Dihydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (14). Yield 65%. Mp > 250 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 6.99 (s, 2H, Ar), 7.47 (s, 1H, Ar), 7.81 (d, J = 8.0 Hz, 1H, BIM), 7.99 (d, J = 8.5 Hz, 1H, BIM), 8.31 (s, 1H, BIM), 9.40 brs (1H, OH); ^{13}C (DMSO-d_6): δ 113.2, 116.4 (2C), 119.8 (2C), 120.5 (2C), 132.7, 139.2, 142.6, 145.7 , 152.0, 154.2, 153.9. IR (Nujol) 31.84, 2720, 1718, 1620 cm^{-1}. *m/z* 271 $(M+H)^+$. Anal. Calcd for $C_{14}H_{10}N_2O_4$: C, 62.22; H, 3.73; N, 10.37. Found: C, 62.28; H, 3.71; N, 10.34.

4.2.1.7. 2-(2,5-Dihydroxyphenyl)-1H-benzo[d]imidazole-5-sulfonic acid (15). Yield 71%. Mp > 250 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.04 (s, 2H, Ar), 7.43 (s, 1H, Ar), 7.75 (s, 2H, Ar), 8.02 (s, 1H, Ar), 9.41 (s, 1H, NH). IR (Nujol) 3392, 3225, 3097, 1633, 1569 cm^{-1}. *m/z* 307 $(M+H)^+$. Anal. Calcd for $C_{13}H_{10}N_2O_5S$: C, 50.98; H, 3.29; N, 9.15. Found: C, 50.94; H, 3.31; N, 9.18.

4.2.1.8. 2-(3,4-Dihydroxyphenyl)-1H-benzo[d]imidazole-5-carbonitrile (16). Yield 75%. Mp > 250 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.04 (d, J = 8.5 Hz, 1H, Ar), 7.65 (s, 1H, Ar), 7.67 (d, J = 8. 5 Hz, 1H, Ar), 7.79 (d, J = 8.5 Hz, 1H, BIM), 7.85 (d, J = 8.0 Hz, 1H, BIM), 8.21 (s, 1H, BIM). IR (Nujol) 3428, 3331, 3105, 2242, 1611 cm^{-1}. *m/z* 252 $(M+H)^+$. Anal. Calcd for $C_{14}H_9N_3O_2$: C, 66.93; H, 3.61; N, 16.73. Found: C, 66.85; H, 3.59; N, 16.70.

4.2.1.9. 2-(3,4-Dihydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (17). Yield 81%. Mp 224–226 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.04 (d, J = 8.5 Hz, 1H, Ar), 7.67 (d, J = 9.0 Hz, 1H, Ar), 7.69 (s, 1H, Ar), 7.78 (d, J = 8.5 Hz, 1H, BIM), 8.01 (d, J = 8.0 Hz, 1H, BIM), 8.23

(s, 1H, BIM), 9.50 (brs, 1H, OH). IR (Nujol) 3317, 2760, 1694, 1603, 1524 cm^{-1}. *m/z* 271 $(M+H)^+$. Anal. Calcd for $C_{14}H_{10}N_2O_4$: C, 62.22; H, 3.73; N, 10.37. Found: C, 62.16; H, 3.74; N, 10.40.

*4.2.1.10. 2-(3-Hydroxy-4-methoxyphenyl)-1H-benzo[d]imidazole-5-carbonitrile (**18**)*. Yield 58%. Mp > 250 °C (EtOH). ^1H NMR (DMSO-d_6): δ 3.81 (s, 3H, OCH_3), 6.66–6.68 (m, 2H, Ar), 7.73 (d, J = 8.0 Hz, 1H, BIM), 7.83 (d, J = 8.5 Hz, 1H, BIM), 8.06 (d, J = 8.5 Hz, 1H, Ar), 8.15 (s, 1H, BIM). IR (Nujol) 2228, 1618 cm^{-1}. *m/z* 266 $(M+H)^+$. Anal. Calcd for $C_{15}H_{11}N_3O_2$: C, 67.92; H, 4.18; N, 15.84. Found: C, 67.98; H, 4.20; N, 15.80.

*4.2.1.11. 2-(3-Hydroxy-4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (**19**)*. Yield 60%. Mp 223–234 °C (EtOH). ^1H NMR (DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 6.72 (s, 2H, Ar), 7.81 (d, J = 8.0 Hz, 1H, Ar), 8.00 (d, J = 8.5 Hz, 1H, BIM), 8.08 (d, J = 9.5 Hz, 1H, BIM), 8.30 (s, 1H, BIM), 9.70 (brs, 1H, OH). IR (Nujol) 3293, 2725, 1697, 1626 cm^{-1}. *m/z* 285 $(M+H)^+$. Anal. Calcd for $C_{15}H_{12}N_2O_4$: C, 63.38; H, 4.25; N, 9.85. Found: C, 63.33; H, 4.27; N, 9.88.

*4.2.1.12. 2-(3-Hydroxy-4-methoxyphenyl)-1H-benzo[d]imidazole-5-sulfonic acid (**20**)*. Yield 69%. Mp > 250 °C (EtOH). ^1H NMR (DMSO-d_6): δ 3.93 (s, 3H, OCH_3), 7.30 (d, J = 8.5 Hz, 1H, Ar), 7.60 (s, 1H, Ar), 7.68–7.68 (m, 2H, Ar and BIM), 7.79 (d, J = 9.0 Hz, 1H, BIM), 7.93 (s, 1H, BIM), 9.78 (s, 1H, OH). IR (Nujol) 3367, 3149, 3089, 2781, 1634, 1610, 1583 cm^{-1}. *m/z* 321 $(M+H)^+$. Anal. Calcd for $C_{14}H_{12}N_2O_5S$: C, 52.49; H, 3.78; N, 8.75. Found: C, 52.54; H, 3.77; N, 8.72.

*4.2.1.13. 2-(3-Ethoxy-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (**21**)*. Yield 52%. Mp > 250 °C (EtOH). ^1H NMR (DMSO-d_6): δ 1.37 (t, J = 7.0 Hz, 3H, CH_3), 4.11 (q, J = 7.0 Hz, 2H, CH_2), 6.98 (t, J = 8.0 Hz, 1H, Ar), 7.15 (d, J = 8.0 Hz, 1H, Ar), 7.64 (d, J = 7.5 Hz, 1H, Ar), 7.77 (d, J = 8.0 Hz, 1H, BIM), 7.95 (d, J = 9.0 Hz, 1H, BIM), 8.27 (s, 1H, BIM). IR (Nujol) 3463, 3030, 2680, 1682, 1624, 1594 cm^{-1}. *m/z* 299 $(M+H)^+$. Anal. Calcd for $C_{16}H_{14}N_2O_4$: C, 64.42; H, 4.73; N, 9.39. Found: C, 64.37; H, 4.74; N, 9.43.

*4.2.1.14. 2-(3-Ethoxy-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-sulfonic acid (**22**)*. Yield 56%. Mp 236–237 °C (EtOH). ^1H NMR (DMSO-d_6): δ 1.40 (t, J = 6.5 Hz, 3H, CH_3), 4.21 (q, J = 6.5 Hz, 2H, CH_2), 7.08 (t, J = 8.0 Hz, 1H, Ar), 7.31 (d, J = 8.0 Hz, 1H, Ar), 7.59 (d, J = 8.0 Hz, 1H, Ar), 7.78 (d, J = 9.0 Hz, 1H, BIM), 7.80 (d, J = 8.5 Hz, 1H, BIM), 8.06 (s, 1H, BIM). IR (Nujol) 3448, 3227, 1625, 1561 cm^{-1}. *m/z* 335 $(M+H)^+$. Anal. Calcd for $C_{15}H_{14}N_2O_5S$: C, 53.88; H, 4.22; N, 8.38. Found: C, 53.94; H, 4.24; N, 8.34.

*4.2.1.15. 2-(2-Hydroxy-4-methoxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (**23**)*. Yield 58%. Mp 219–220 °C (EtOH). ^1H NMR (DMSO-d_6): δ 3.80 (s, 3H, OCH_3), 7.22 (d, J = 8.5 Hz, 1H, Ar), 7.65 (s, 1H, Ar), 7.70 (d, J = 8.5 Hz, 1H, Ar), 7.76 (d, J = 8.0 Hz, 1H, BIM), 7.98 (d, J = 8.5 Hz, 1H, BIM), 8.22 (s, 1H, BIM). IR (Nujol) 3447, 3130, 2766, 1713, 1615 cm^{-1}. *m/z* 285 $(M+H)^+$. Anal. Calcd for $C_{15}H_{12}N_2O_4$: C, 63.38; H, 4.25; N, 9.85. Found: C, 63.45; H, 4.23; N, 9.89.

*4.2.1.16. 2-(2-Hydroxy-4-methoxyphenyl)-1H-benzo[d]imidazole-5-sulfonic acid (**24**)*. Yield 59%. Mp 226–227 °C (EtOH). ^1H NMR (DMSO-d_6): δ 3.87 (s, 3H, OCH_3), 6.69 (s, 1H, Ar), 6.78 (m, 1H, Ar), 7.71 (m, 2H, BIM), 7.98 (m, 1H, Ar), 8.02 (s, 1H, BIM). IR (Nujol) 3163, 1621, 1572 cm^{-1}. *m/z* 321 $(M+H)^+$. Anal. Calcd for $C_{14}H_{12}N_2O_5S$: C, 52.49; H, 3.78; N, 8.75. Found: C, 52.44; H, 3.80; N, 8.78.

*4.2.1.17. 2-(5-Chloro-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-carbonitrile (**25**)*. Yield 83%. Mp 200–202 °C (EtOH). ^1H NMR (DMSO-d_6): δ 7.16 (d, J = 9.0 Hz, 1H, Ar), 7.47 (d, J = 9.0 Hz, 1H, Ar), 7.71 (d, J = 8.5 Hz, 1H, BIM), 7.86 (d, J = 8.5 Hz, 1H, BIM), 8.19 (s, 1H, Ar), 8.20 (s, 1H, BIM). IR (Nujol) 3340, 3059, 2727, 2232, 1615 cm^{-1}. *m/z* 270 $(M+H)^+$. Anal. Calcd for $C_{14}H_8ClN_3O$: C, 62.35; H, 2.99; N, 15.58. Found: C, 62.42; H, 2.98; N, 15.55.

*4.2.1.18. 2-(5-Chloro-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (**26**)*. Yield 82%. Mp 203–204 °C (EtOH). ^1H NMR (DMSO-d_6): δ 7.14 (d, J = 8.5 Hz, 1H, Ar), 7.45 (d, J = 8.0 Hz, 1H, Ar), 7.78 (d, J = 8.5 Hz, 1H, BIM), 7.95 (d, J = 8.5 Hz, 1H, BIM), 8.19 (s, 1H, Ar), 8.28 (s, 1H, BIM). IR (Nujol) 3320, 3225, 3070, 2688, 1685, 1632, 1558 cm^{-1}. *m/z* 289 $(M+H)^+$. Anal. Calcd for $C_{14}H_9ClN_2O_3$: C, 58.25; H, 3.14; N, 9.70. Found: C, 58.16; H, 3.15; N, 9.74.

*4.2.1.19. 2-(5-Chloro-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-sulfonic acid (**27**)*. Yield 85%. Mp 226–227 °C (EtOH). ^1H NMR (DMSO-d_6): δ 7.25 (d, J = 8.0 Hz, 1H, Ar), 7.61 (d, J = 8.0 Hz, 1H, Ar), 7.80 (m, 2H, BIM and Ar), 8.06 (s, 1H, BIM), 8.20 (s, 1H, BIM). IR (Nujol) 3582, 3375, 3060, 2720, 1624, 1556 cm^{-1}. *m/z* 325 $(M+H)^+$. Anal. Calcd for $C_{13}H_9ClN_2O_4S$: C, 48.08; H, 2.79; N, 8.63. Found: C, 48.03; H, 2.81; N, 8.67.

*4.2.1.20. 2-(5-Bromo-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-carbonitrile (**28**)*. Yield 85%. Mp 215–216 °C (EtOH). ^1H NMR (DMSO-d_6): δ 7.12 (d, J = 9.0 Hz, 1H, Ar), 7.58 (d, J = 9.0 Hz, 1H, Ar), 7.72 (d, J = 8.0 Hz, 1H, BIM), 7.86 (d, J = 8.5 Hz, 1H, BIM), 8.20 (s, 1H, Ar), 8.31 (s, 1H, BIM). IR (Nujol) 2220, 1607, 1552 cm^{-1}. *m/z* 314 $(M+H)^+$. Anal. Calcd for $C_{14}H_8BrN_3O$: C, 53.53; H, 2.57; N, 13.38. Found: C, 53.49; H, 2.58; N, 13.41.

*4.2.1.21. 2-(5-Bromo-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (**29**)*. Yield 84%. Mp 233–235 °C (EtOH). ^1H NMR (DMSO-d_6): δ 7.09 (d, J = 8.5 Hz, 1H, Ar), 7.60 (d, J = 8.5 Hz, 1H, Ar), 7.78 (d, J = 8.5 Hz, 1H, BIM), 7.95 (d, J = 8.5 Hz, 1H, BIM), 8.28 (s, 1H, Ar), 8.31 (s, 1H, BIM). IR (Nujol) 3320, 3170, 2670, 1638, 1624, 1555 cm^{-1}. *m/z* 332 $(M+H)^+$. Anal. Calcd for $C_{14}H_9BrN_2O_3$: C, 50.47; H, 2.72; N, 8.41. Found: C, 50.53; H, 2.71; N, 8.44.

*4.2.1.22. 2-(5-Bromo-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-sulfonic acid (**30**)*. Yield 88%. Mp 240–241 °C (EtOH). ^1H NMR (DMSO-d_6): δ 7.16 (d, J = 9.0 Hz, 1H, Ar), 7.72 (d, J = 9.0 Hz, 1H, Ar), 7.79 (m, 2H, BIM), 8.04 (s, 1H, Ar), 8.27 (s, 1H, BIM). IR (Nujol) 3350, 3264, 3199, 2722, 2670, 1625, 1605, 1556 cm^{-1}. *m/z* 368 $(M+H)^+$. Anal. Calcd for $C_{13}H_9BrN_2O_4S$: C, 42.29; H, 2.46; N, 7.59. Found: C, 42.24; H, 2.45; N, 7.62.

*4.2.1.23. 2-(2,3,4-Trihydroxyphenyl)-1H-benzo[d]imidazole-5-carbonitrile (**31**)*. Yield 72%. Mp 232–233 °C (EtOH). ^1H NMR (DMSO-d_6): δ 6.65 (d, J = 9.0 Hz, 1H, Ar), 7.60 (d, J = 8.5 Hz, 1H, Ar), 7.80 (d, J = 8.0 Hz, 1H, BIM), 7.88 (d, J = 8.0 Hz, 1H, BIM), 8.20 (s, 1H, BIM). IR (Nujol) 2228, 1619, 1571 cm^{-1}. *m/z* 267 $(M+H)^+$. Anal. Calcd for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N, 15.72. Found: C, 62.85; H, 3.41; N, 15.76.

*4.2.1.24. 2-(2,4,6-Trihydroxyphenyl)-1H-benzo[d]imidazole-5-carbonitrile (**34**)*. Yield 74%. Mp > 250 °C (EtOH). ^1H NMR (DMSO-d_6): δ 6.22 (s, 1H, Ar), 6.22 (s, 1H, Ar), 7.87 (d, J = 8.5 Hz, 1H, BIM), 7.95 (d, J = 8.5 Hz, 1H, BIM), 8.21 (s, 1H, BIM). IR (Nujol) 2230, 1625 cm^{-1}. *m/z* 267 $(M+H)^+$. Anal. Calcd for $C_{14}H_9N_3O_3$: C, 62.92; H, 3.39; N, 15.72. Found: C, 62.99; H, 3.38; N, 15.69.

*4.2.1.25. 2-(2,4,6-Trihydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (**35**)*. Yield 75%. Mp > 250 °C (EtOH). ^1H NMR (DMSO-d_6): δ 6.01 (s, 2H, Ar), 7.69 (d, J = 8.0 Hz, 1H, BIM), 7.83 (d, J = 8.5 Hz, 1H, BIM), 8.26 (s, 1H, BIM), 13.19 (s, 1H, OH), 13.24 (s, 1H, OH). IR (Nujol) 3404, 3233, 2718, 1704, 1614, 1565 cm^{-1}. *m/z* 287 $(M+H)^+$.

Anal. Calcd for $C_{14}H_{10}N_2O_5$: C, 58.74; H, 3.52; N, 9.79. Found: C, 58.69; H, 3.50; N, 9.83.

*4.2.1.26. 2-(2,4,6-Trihydroxyphenyl)-1H-benzo[d]imidazole-5-sulfonic acid (**36**)*. Yield 75%. Mp 224–225 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 6.14 (s, 2H, Ar), 7.70 (d, J = 8.5 Hz, 1H, BIM), 7.74 (d, J = 8.5 Hz, 1H, BIM), 8.11 (s, 1H, BIM), 10.45 (s, 1H, NH), 11, 80 (s, 2H, OH), 13,20 (s, 1H, OH). IR (Nujol) 3489, 3216, 1629 cm^{-1}. *m/z* 267 (M + H)$^+$. Anal. Calcd for $C_{13}H_{10}N_2O_6S$: C, 48.45; H, 3.13; N, 8.69. Found: C, 48.49; H, 3.11; N, 8.72.

*4.2.1.27. 2-(4-(Diethylamino)-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-carboxylic acid (**37**)*. Yield 59%. Mp 212–213 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 1.14 (t, J = 7.0 Hz, 6H, CH_3), 3.41 (q, J = 7.0 Hz, 4H, CH), 6.30 (s, 1H, Ar), 6.47 (d, J = 9.0 Hz, 1H, Ar), 7.70 (d, J = 8.5 Hz, 1H, BIM), 7.85 (d, J = 8.5 Hz, 1H, BIM), 7.94 (d, J = 8.0 Hz, 1H, Ar), 8.20 (s, 1H, BIM). ^{13}C (DMSO-d_6): δ 15.6 (2C), 52.1 (2C), 98.6, 106.3, 108.2, 117.2 (2C), 121.3, 128.2 (2C), 131.8, 140.2, 145.2 (2C), 147.6, 163.3. IR (Nujol) 3446, 3181, 2681, 1691, 1615 cm^{-1}. *m/z* 326 (M + H)$^+$. Anal. Calcd for $C_{18}H_{19}N_3O_3$: C, 66.45; H, 5.89; N, 12.91. Found: C, 66.52; H, 5.86; N, 12.94.

*4.2.1.28. 2-(4-(Diethylamino)-2-hydroxyphenyl)-1H-benzo[d]imidazole-5-sulfonic acid (**38**)*. Yield 63%. Mp 221–222 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 1.16 (t, J = 7.0 Hz, 6H, CH_3), 3.43 (q, J = 7.0 Hz, 4H, CH_2), 6.36 (s, 1H, Ar), 6.53 (d, J = 8.5 Hz, 1H, Ar), 7.65 (d, J = 8.5 Hz, 1H, BIM), 7.71 (d, J = 8.5 Hz, 1H, BIM), 7.85 (d, J = 8.5 Hz, 1H, Ar), 7.97 (s, 1H, BIM), 11.42 (s, 1H, NH), 13.55 (s, 1H, OH). ^{13}C (DMSO-d_6): δ 14.1 (2C), 45.3 (2C), 100.3, 104.1, 110.8, 112.3, 115.6, 122.7, 133.2, 139.7, 143.7, 145.6 (2C), 155.2, 157.5. IR (Nujol) 3442, 3321, 1613 cm^{-1}. *m/z* 362 (M + H)$^+$. Anal. Calcd for $C_{17}H_{19}N_3O_4S$: C, 56.50; H, 5.30; N, 11.63. Found: C, 56.45; H, 5.28; N, 11.67.

*4.2.1.29. 2-(2-Hydroxynaphthalen-1-yl)-1H-benzo[d]imidazole-5-carboxylic acid (**39**)*. Yield 58%. Mp > 250 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.42 (m, 2H, Ar), 7.54 (t, J = 8.5 Hz, 1H, Ar), 7.87 (d, J = 9.0 Hz, 1H, BIM), 7.92–7.96 (m, 2H, Ar), 8.05 (d, J = 8.5 Hz, 1H, BIM), 8.10 (d, J = 8.5 Hz, 1H, Ar), 8.35 (s, 1H, BIM). IR (Nujol) 3463, 2680, 1682, 1624, 1594 cm^{-1}. *m/z* 305 (M + H)$^+$. Anal. Calcd for $C_{18}H_{12}N_2O_3$: C, 71.05; H, 3.97; N, 9.21. Found: C, 70.98; H, 3.98; N, 9.25.

*4.2.1.30. 2-(2-Hydroxynaphthalen-1-yl)-1H-benzo[d]imidazole-5-sulfonic acid (**40**)*. Yield 61%. Mp > 250 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 7.43 (d, J = 9.0 Hz, 1H, Ar), 7.47 (t, J = 7.5 Hz, 1H, Ar), 7.57 (t, J = 8.0 Hz, 7.5 Hz, 1H, Ar), 7.72 (d, J = 8.0 Hz, 1H, Ar), 7.83 (d, J = 9.0 Hz, 1H, Ar), 7.89 (d, J = 8.0 Hz, 1H, Ar) 8.01 (d, J = 8.0 Hz, 1H, BIM), 8.04 (s, 1H, BIM), 8.18 (d, J = 8.5 Hz, 1H, BIM), 10.81 (s. 1H, OH). IR (Nujol) 3423, 1621, 1588 cm^{-1}. *m/z* 341 (M + H)$^+$. Anal. Calcd for $C_{17}H_{12}N_2O_4S$: C, 59.99; H, 3.55; N, 8.23. Found: C, 60.05; H, 3.53; N, 8.26.

*4.2.2. (E)-Methyl 4-amino-3-(3-(4-hydroxyphenyl)acrylamido)benzoate (**43**)*

A solution of 4-hydroxycinnamic acid (**41**) (0.33 g, 2 mmol), EDC (0.38 g, 2.2 mmol) and HOBt (0.26 g, 2 mmol) in anhydrous MeCN (10 mL) was stirred at r.t. for 30 min, then methyl 3,4-diaminobenzoate (**42**) (0.33 g, 2 mmol) was added. The mixture was stirred at r.t. After 36 h the solvent was removed in vacuum. The residue was dissolved in ethyl acetate (AcOEt) (20 mL) and washed sequentially with brine (2 × 5 mL), 10% citric acid (2 × 5 mL), saturated $NaHCO_3$ aqueous solution (2 × 5 mL) and water (2 × 5 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated under vacuum to give the title compound in 82% yield. Mp 174–176 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 3.76 (s, 3H, CH_3), 5.81 (s, 2H, NH_2), 6.67 (d, J = 16.0 Hz, 1H, CH), 6.76 (d, J = 8.0 Hz, 1H, Ar), 6.83 (d, J = 9.0 Hz, 2H, Ar), 7.46–7.55 (m, 5H, Ar and CH) 8.03 (s, 1H, Ar), 9.26 (s, 1H, NH), 9.92 (s, 1H, OH). IR (Nujol) 3148, 1700, 1599 cm^{-1}. *m/z* 313 (M + H)$^+$. Anal. Calcd for $C_{17}H_{16}N_2O_4$: C, 65.38; H, 5.16; N, 8.97. Found: C, 65.32; H, 5.18; N, 9.00.

*4.2.3. (E)-Methyl 2-(4-hydroxystyryl)-1H-benzo[d]imidazole-5-carboxylate (**44**)*

(*E*)-methyl 4-amino-3-(3-(4-hydroxyphenyl)acrylamido)benzoate (**43**) (0.70 g, 2.2 mmol) was dissolved in ethanol (1.5 mL) and then 6 N aqueous hydrochloric acid solution (15 mL) was added. The mixture was heated at 100 °C for 5 h The ethanol was removed in vacuum and the residue cooled to 10 °C. The formed precipitate was filtered off and air dried. Yield 74%. Mp 215–216 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 3.92 (s, 3H, CH_3), 6.93 (d, J = 7.5 Hz, 2H, Ar), 7.09 (d, J = 15.5 Hz, 1H, CH), 7.61 (d, J = 7.5 Hz, 2H, Ar), 7.82 (s, 1H, Ar), 8.05 (m, 2H, Ar), 8.18 (d, J = 15.5 Hz, 1H, CH), 8.23 (s, 1H, NH), 10.34 (s, 1H, OH). IR (Nujol)3453, 3223, 1621, 1588 cm^{-1}. *m/z* 295 (M + H)$^+$. Anal. Calcd for $C_{17}H_{14}N_2O_3$: C, 69.38; H, 4.79; N, 9.52. Found: C, 69.43; H, 4.76; N, 9.56.

*4.2.4. (E)-2-(4-hydroxystyryl)-1H-benzo[d]imidazole-5-carboxylic acid (**45**)*

To a solution of (*E*)-methyl 2-(4-hydroxystyryl)-1*H*-benzo[*d*]imidazole-5-carboxylate (**44**) (0.36 g, 1.22 mmol) in ethanol (5 mL) 2.5 N aqueous solution sodium hydroxide (2.5 mL) was added. The mixture was stirred at r.t per 24 h. The ethanol was removed *in vacuo*. The residue was ice added and hydrochloric acid 10% solution added until pH 6. The obtained precipitate was filtered off, washed with cold water (3 × 10 mL) and dried. Yield 48%. Mp > 250 °C (EtOH). ^{1}H NMR (DMSO-d_6): δ 6.89 (d, J = 7.5 Hz, 2H, Ar), 7.04 (d, J = 15.5 Hz, 1H, CH), 7.55 (d, J = 7.5 Hz, 2H, Ar), 7.75 (s, 1H, Ar), 7.99 (m, 2H, Ar), 8.16 (d, J = 15.5 Hz, 1H, CH), 10.30 (s, 1H, OH). ^{13}C (DMSO-d_6): δ 116.3, 117.6 (2C), 119.4, 125.3, 126.2 (2C), 130.7, 131.3 (2C), 136.3, 141.2, 143.5, 144.7, 161.4, 169.8. IR (Nujol) 3345, 3190, 1686, 1639, 1600 cm^{-1}. *m/z* 281 (M + H)$^+$. Anal. Calcd for $C_{16}H_{12}N_2O_3$: C, 68.56; H, 4.32; N, 9.99. Found: C, 68.50; H, 4.30; N, 10.03.

4.3. Biological assays

4.3.1. DPPH assay

The activity of the synthesized derivatives against DPPH$^\bullet$ radical was tested following the Wang et al method [25] modified as previously reported [17]. The results are expressed as μmol TE/g corresponding to an inhibition of the radical equal to 50%, except for some compounds.

4.3.2. FRAP test

The FRAP antioxidant capacity of benzimidazoles was measured according to the method described previously [26], reading the absorbance of the reaction mixture at 593 nm. The values are expressed as μmol TE/g compound.

4.3.3. Oxygen radical absorbance capacity (ORAC)

Peroxyl radical scavenging activity was measured based on a protocol previously reported and modified [27]. Phosphate buffer solution (pH 7.4) was used to prepare sample solutions and a series of Trolox solutions (40–240 μM) by diluting a 2 mM solution of Trolox. 25 μl of sample solution, Trolox dilution or phosphate buffer solution (pH 7.4) used as blank was placed in wells of a 96-well black microplate (VWR). Measurements of fluorescence were carried out at 37 °C and recorded at 5 min intervals up 30 min after the addition of AAPH. The ORAC values were calculated according to the method of Cao et al. [28] and expressed as Trolox equivalents (μmol TE/μmol).

4.3.4. Evaluation of filtering parameters

The method followed is an adaptation of the official method for determining the value of SPF *in vitro* [29]. Solutions of the tested

benzimidazoles were prepared in MeOH at a concentration of 0.000034 (± 0.0000033) M and the absorption spectra were recorded. To calculate the SPF value *in vitro*, the absorbance values obtained were transformed into transmittance values, using the equation below:

$$A(\lambda) = \mathrm{Log}[T(\lambda)]$$

The transmittance spectrum was elaborated with the SPF calculator software (version 2.1, Shimadzu, Milan, Italy) to obtain the values of SPF, UVA/UVB, UVAPF and λ critical.

4.3.5. Formulations

Formulation A and Formulation B, standard oil/water emulsions, were prepared for Oxisol and benzimidazole **15**. Each phase, after adding the individual ingredients, was heated to a temperature between 60 and 70 °C. At this point, below mechanical stirring, the oily phase was added to the aqueous phase. The selected compound was added in the subsequent cooling phase of the emulsion, kept constant agitation, to preserve it from possible thermal degradation. The emulsion, once cooled to 25 °C, is stored in the refrigerator until the next day of analysis. The formulation compositions are the following:

Formulation A: Aqua, Ethylhexyl Stearate, Tribehenin PEG-20 Esters, Butyrospermum Parkii, Olea Europaea Oil Unsaponifiables, Oxisol or benzimidazole **15**, Xanthan Gum, Caprylic/Capric Triglyceride, Cetearyl Alcohol, Dicaprylyl Carbonate.

Formulation B: Aqua, Cetearyl Alcohol, Tribehenin PEG-20 Esters, Butyrospermum Parkii, Olea Europaea Oil Unsaponifiables, Oxisol or benzimidazole **15**, Xanthan Gum, Caprylic/Capric Triglyceride.

4.3.6. Franz cells apparatus

Cuprophan regenerated cellulose from Medicell (London, UK), membrane thickness (11.5 μm ± 0.5 μm), molecular weight cut off range (10,000 Da) was chosen as synthetic membrane employed in Franz diffusion cells experiments in this study. The effective diffusion area of the Franz cells was 0.6 cm^2 and receptor volume was 4.3 mL. Before being mounted on the diffusion cells, the membranes were conditioned with the receiving solution (DMSO). The membrane was positioned between the two compartments and the formulation (0.5 g) was placed in the donor compartment, in contact with the membrane. The aforementioned compartment was then sealed with Parafilm in order to avoid loss of formulation components. The thus prepared Franz cells were immersed in a thermostat bath to guarantee a surface temperature of the membrane of 37 ± 1 °C, keeping the receiving solvent under continuous magnetic stirring. At each set time (after 30 min, 1 h and then every hour thereafter up to 6 h) using a glass syringe, 500 μl of sample were taken and replaced by 500 μl of fresh receiving medium, and the absorbance of the samples was measured using a UV–VIS spectrophotometer at the λmax. A calibration line and a release curve were built for each compound.

4.3.7. Growth inhibition assays

Cell growth inhibition assays were carried out using two human cell lines, melanoma Colo38 [30,31,17] and keratinocyte HaCat [31,32], cells. Colo 38 cell line was maintained in RPMI 1640, supplemented with 10% fetal bovine serum (FBS), penicillin (100 Units mL-1), streptomycin (100 μg mL-1) and glutamine (2 mM) (complete medium); the pH of the medium was 7.2 and the incubation was performed at 37 °C in a 5% CO_2 atmosphere. HaCat cell line were grown in DMEM medium (4.5 g/L glucose) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin/streptomycin and 2 mM L-glutamine, in a 5% CO_2 environment at 37 °C. Benzimidazole derivatives were dissolved in MeOH/DMSO 10% to obtain 20 mM stock solutions and diluted before cell treatment in MeOH 100%. The tested benzimidazoles were added at serial dilutions to the cell cultures and incubated for 3 days. Cells were then harvested, suspended in physiological solution and counted with a Z2 Coulter Counter (Coulter Electronics, Hialeah, FL, USA). The cell number/ml was determined as IC_{50} after 3 days of culture, when untreated cells are in log phase of cell growth. Untreated cells were placed in every plate as negative control.

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Declaration of Competing Interest

The authors declare no conflict of interest.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2019.103396.

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